TRANSMITTAL OF APPEAL BRIEF (Large Entity)	Docket No. 112843-032

In Re Application Of: **Reniero et al.**

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/936,542	Sept. 10, 2001	D. Ware	29157	1651	7122

Invention: **LACTOBACILLUS STRAINS CAPABLE OF PREVENTING DIARRHOEA CAUSED BY PATHOGENIC BACTERIA AND ROTAVIRUSES**

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on

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Dated: **October 4, 2004**

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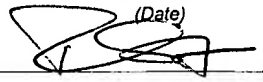

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AF/1651

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant(s): Reniero et al.
Appl. No.: 09/936,542
Conf. No.: 7122
Filed: September 10, 2001
Title: LACTOBACILLUS STRAINS CAPABLE OF PREVENTING DIARRHOEA CAUSED BY PATHOGENIC BACTERIA AND ROTAVIRUSES
Art Unit: 1651
Examiner: D. Ware
Docket No.: 112843-032

Commissioner for Patents
P.O. Box 1450
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APPELLANTS' APPEAL BRIEF

Dear Sir:

This Appeal Brief is submitted in support of the Notice of Appeal submitted by Appellants on August 4, 2004 in the above-identified patent application. This Appeal is taken from the Final Rejection dated April 6, 2004.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A. by virtue of an Assignment recorded at the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellants do not believe there are any known appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF THE CLAIMS

Claims 1, 2 and 4-22 are pending in this application. A copy of appealed Claims 1, 2 and 4-22 is attached in the appendix. In the Final Office dated April 6, 2003, claims 1, 2 and 4-22

are rejected under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over EP 0861905. A copy of the Final Office Action is appended hereto as Exhibit A of the Supplemental Appendix and a copy of the cited reference is appended hereto as Exhibit B of the Supplemental Appendix.

Appellants note that Claims 1, 2 and 4-22 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting. As the obviousness-type double patenting rejections in view of Claims 1, 2 and 4-22 of co-pending application no. 09/936,489 and co-pending application no. 09/936,453 are provisional, Appellants have asserted that they plan to submit a terminal disclaimer, if necessary, to overcome the provisional rejections once either one or both of the co-pending applicants have granted. Therefore, Appellants believe that they have been responsive to the provisional rejections at this stage in the prosecution.

IV. STATUS OF THE AMENDMENTS

An Amendment After Final was filed. More specifically, Claim 2 was amended to address a rejection under 35 U.S.C. § 112, second paragraph. The Patent Office issued an advisory action on July 26, 2004 and indicated that the amendment was entered and that the rejection under 35 U.S.C. § 112, second paragraph was withdrawn. A copy of the advisory action is attached hereto as exhibit C.

V. SUMMARY OF THE INVENTION

The present invention pertains to microorganisms of the genus Lactobacillus, that are useful in preventing diarrhoea brought about by both pathogenic bacteria and rotaviruses, respectively. In particular, the present invention relates to the use of the microorganisms for the preparation of an ingestable support and to a composition containing the same. (Specification, p. 1, lines 3-7).

Organisms that produce lactic acid as a major metabolic component have been known for a long time. These bacteria may be found in milk or in milk processing factories, respectively, living or decaying plants but also in the intestine of man and animals. These microorganisms, summarized under the term "lactic acid bacteria", represent a rather inhomogeneous group and

include, for example, the genera Lactococcus, Lactobacillus, Streptococcus, Bifidobacterium, Pediococcus, and the like. (Specification, p. 1, lines 8-13).

Lactic acid bacteria have been utilized as fermenting agents for the preservation of food taking benefit of a low pH and the action of fermentation products generated during the fermentative activity thereof to inhibit the growth of spoilage bacteria. To this end, lactic acid bacteria have been used for preparing a variety of different foodstuff such as cheese, yogurt and other fermented diary products from milk. (Specification, p. 1, lines 14-18).

Lactic acid bacteria have attracted a great deal of attention in that some strains have been found to exhibit valuable properties to man and animals upon ingestion. In particular, specific strains of the genus Lactobacillus or Bifidobacterium have been found to be able to colonize the intestinal mucosa and to assist in the maintenance of the well-being of man and animal. (Specification, p. 1, lines 19-23).

Research has also focused on the potential use of lactic acid bacteria as probiotic agents. Probiotics are considered to be viable microbial preparations which promote the individual's health by preserving the natural microflora in the intestine. A microbial preparation may be commonly accepted as a probiotic in case the effectual microbes thereof and their mode of action are known. Probiotics are deemed to attach to the intestine's mucosa, colonize the intestinal tract and likewise prevent attachment of harmful microorganisms thereon. A crucial prerequisite for their action resides in that they have to reach the gut's mucosa in a proper and viable form and are not destroyed in the upper part of the gastrointestinal tract, especially by the influence of the low pH prevailing in the stomach. (Specification, p. 2, lines 4-13).

In knowledge of the valuable properties particular strains of lactic acid bacteria may provide, there is a desire in the art for additional lactic acid bacterial strains that are beneficial to the well being of man and/or animal. Consequently, a problem of the present invention is to provide additional bacterial strains that exhibit new properties beneficial for man and/or animals. (Specification, p. 3, lines 4-8).

As previously discussed, the present invention provides microorganisms, namely lactic acid bacteria, belonging to the genus Lactobacillus having the traits of being capable of preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses. According to a preferred

embodiment, the Lactobacillus strains are capable of adhering to the intestinal mucosa of a host organism and are capable of essentially colonizing it. (Specification, p. 3, lines 9-14).

According to yet another preferred embodiment, the Lactobacillus strains are capable to grow in the presence of up to 0.4 % bile salts, so that they may easily pass the gastrointestinal tract and remain essentially active. (Specification, p. 3, lines 15-17).

According to another preferred embodiment, the lactic acid bacterium includes Lactobacillus rhamnosus or Lactobacillus paracasei, preferably Lactobacillus paracasei, and is more preferably Lactobacillus paracasei CNCM I-2116. (Specification, p. 3, lines 18-20).

The microorganisms of the present invention have been shown to exhibit such properties as follows. They are gram positive, catalase negative, NH_3 form arginine negative and CO_2 production negative. They also produce L(+) lactic acid, are capable to grow in the presence of bile salts in a concentration of up to about 0.4 % and may essentially prevent infection of epithelial cells by rotaviruses. (Specification, pg. 3, lines 18-22).

The microorganisms of the present invention may be used for the preparation of a variety of ingestable support materials, such as milk, yogurt, curd, fermented milks, milk based fermented products, fermented cereal based products, milk based powders, infant formulae and may be included in the support in an amount of from about 10^5 cfu / g to about 10^{11} cfu / g. The present invention also provides a food or a pharmaceutical composition containing at least one of the Lactobacillus strains having the above traits. (Specification, p. 4, lines 1-9).

For preparing a food composition according to the present invention, at least one of the Lactobacillus strains is incorporated in a suitable support, for example, in an amount of from about 10^5 cfu / g to about 10^{11} cfu / g, preferably from about 10^6 cfu / g to about 10^{10} cfu / g, more preferably from about 10^7 cfu / g to about 10^9 cfu / g. (Specification, p. 4, lines 10-14).

In case of a pharmaceutical preparation, the product may be prepared in forms of tablets, liquid bacterial suspensions, dried oral supplements, wet oral supplements, dry tube feeding, wet tube feeding and the like with the amount of Lactobacillus strains to be incorporated therein in the range of up to 10^{12} cfu / g, preferably from about 10^7 cfu / g to about 10^{11} cfu / g, more preferably from about 10^7 cfu / g to about 10^{10} cfu / g. (Specification, p. 4, lines 18-19).

The activity of the microorganisms in the individual's intestine is naturally dose dependent. In this regard, the more the microorganisms are incorporated by ingesting the above

food material or the pharmaceutical composition the higher the protective and/or curing activity of the microorganisms. Since the novel microorganisms are not detrimental to mankind and animals and have eventually been isolated from baby feces a high amount thereof may be incorporated so that essentially a high proportion of the individual's intestine will be colonized by the microorganisms as claimed. (Specification, p. 4, lines 20-26).

Appellants have investigated baby feces and isolated a variety of different bacterial strains therefrom. These strains were subsequently examined for their capability to prevent infection of epithelial cells with rotaviruses and pathogenic bacteria known to cause diarrhoea.

Several bacterial genera including Lactobacillus, Lactococcus, Streptococcus were screened for their inhibitory properties. The tests were essentially performed with three rotavirus serotypes representing the major etiological agents of human viral diarrhoea (serotypes GI, G3 and G4) and with pathogenic E. coli and salmonella typhimurium as representatives for pathogenic microorganisms causing diarrhoea in an affected individual. (Specification, p. 6, lines 1-10).

The various lactic acid bacteria were grown in a suitable medium, such as MRS, Hugo-Jago or M17 medium at temperatures of from about 30 to 40°C corresponding to their optimal growth temperature. After reaching stationary growth, the bacteria were collected by centrifugation and resuspended in physiological NaCl solution. Between the different tests the bacterial cells were stored frozen (-20°C). (Specification, p. 6, lines 11-15).

The various rotavirus stocks were prepared by infection of confluent cell monolayers. The rotaviruses were incubated before infection. The cells were infected with 20 tissue culture infectious doses. For assessing anti-rotaviral properties two different protocols were applied. According to one protocol the various bacterial strains were examined for their direct interaction with the rotavirus while in the second protocol the bacteria were screened for those strains that interact with cellular rotavirus receptors. The first protocol involved contacting the respective bacterial suspension each with a different rotavirus strain and incubating in suitable media. Subsequently, the virus-bacterium mixture was applied to a monolayer of cells of the human undifferentiated colon adenoma cells HT-29 and incubation was continued. Virus replication was then assayed. The second protocol involved incubating the respective bacterial suspension first together with a monolayer of cells of the human undifferentiated colon adenoma cells HT-29 and

adding the virus subsequently. After continued incubation virus replication was assayed. Rotavirus replication may easily be assessed by histo-immunological staining of rotavirus proteins in infected cells. A rotavirus inhibitory effect was attributed to a given bacterium when the number of infected cells was reduced by 90% in the cell culture inoculated with rotavirus plus the indicated bacteria in comparison with cells inoculated only with rotavirus. (Specification, p. 6, line 17 to p. 7, line 5).

Out of a total of 260 different bacterial strains primarily isolated merely 9 could be shown to essentially inhibit rotaviral replication. The different bacteria were ascertained to belong to the genus Lactobacillus subspecies rhamnosus or paracasei. One strain, termed Lactobacillus casei ST11, that has been deposited in accordance with the Budapest Treaty and has received the deposit numbers NCC 2461 (I-2116), has been shown to be extremely effective in preventing infection of human cells by rotavirus. Moreover, this particular strain shows excellent growing properties as may be shown by acidification in different media. The strain also shows good performance as regards the survival rate during storage at low temperatures of about 10 °C, which makes it an excellent candidate for being included in food stuff or pharmaceutical compositions to be stored at refrigerator conditions. (Specification, p. 7, lines 6-16.)

For assessing anti-bacterial properties the following approaches were chosen. According to one protocol cultured Lactobacillus strains of the present invention were examined for their capability to prevent adhesion of pathogenic bacteria causing diarrhoea to intestinal cells or invasion thereof into intestinal cells, respectively. To this end, intestinal cells were contacted with the pathogenic bacteria and the cultured Lactobacillus strains of the present invention, and the rate of adhesion, or invasion, respectively, was assessed. According to a second protocol the supernatant of a cell culture of the Lactobacillus strains of the present invention was added together with the pathogenic microorganisms to the intestinal cells and the rate of adhesion, or invasion, respectively, was assessed. During the experimentation it could be shown that the cultured Lactobacilli and the supernatant were effective to prevent both adhesion to and invasion into the intestinal cells indicating that metabolic compounds secreted by the claimed microorganisms are likely responsible for the anti-diarrhoea activity as regards pathogenic bacteria. (Specification, p. 7, lines 18-30).

Appellants' investigations and results thereof with respect to the claimed invention are provided in further detail in Examples 1-11 on pages 11-21. The lactic acid bacterium strains as claimed provide advantages in addition to treating and/or preventing diarrhea caused by rotaviruses and/or pathogenic bacteria as detailed on pages 8-10 of the specification. For example, the claimed strains also exhibit anti-allergenic properties in that the strains have an impact on the synthesis of different immunological mediators. (Specification, p. 8, lines 1-3).

VI. ISSUES

Would the biologically pure culture of lactic acid bacterium strain, method for preparing an ingestible support material using same, a method for treatment, a pharmaceutical composition, a method for preventing a disorder, and a food as defined by Claims 1, 2 and 4-22 have been novel, or in the alternative, not obvious in view of EP 0861905 A1?

VII. GROUPING OF THE CLAIMS

Appellants argue for the separate patentability of each of the independent claims separate and apart from each other set forth in detail below pursuant to the requirements of 37 C.F.R. § 1.192(7), unless otherwise specified.

VIII. ARGUMENT

A. The Claimed Invention -- Independent Claims

On appeal, Claims 1, 7, 9, 11, 12, 20 and 21 are the sole independent claims. Independent Claims 1, 7, 9, 11, 12, 20, and 21 are provided below as follows:

Claim 1 recites a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses wherein the lactic acid bacterium strain is capable of growing in presence of up to 0.4% bile salts.

Claim 7 recites a method for preparing an ingestable support material including using a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 9 recites a method for preparing an ingestable support material including using a supernatant of a biologically pure culture of a lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 11 recites a method for treatment of a disorder associated with diarrhoea including administering to a patient having the disorder associated with diarrhoea a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 12 recites a pharmaceutical composition containing a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses or a supernatant of a culture thereof.

Claim 20 recites a method for preventing a disorder associated with diarrhoea in a patient at risk of same including administering a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 21 recites a food including a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses or a supernatant of a culture thereof.

B. The Rejection

Claims 1, 2 and 4-22 have been rejected under 35 U.S.C. § 102 or, in the alternative, under U.S.C. § 103 in view of EP0861905. The Patent Office essentially asserts that the cited art discloses or suggests each of the features of the claimed invention. In this regard, the Patent Office has relied on a sole reference in support of the anticipation or the alternative obviousness rejections.

C. Claims 1, 2 and 4-22 are Novel and Nonobvious

Appellants respectfully submit that the rejections under 35 U.S.C. § 102 or alternatively under § 103 should be reversed based on the fact that the Patent Office has failed to establish a *prima facie* case of anticipation and obviousness. Appellants submit that the sole reference fails to disclose or suggest the claimed invention.

1. The Applicable Law

"Under 35 U.S.C. § 102, anticipation requires that each and every element of the claimed invention be disclosed in the prior art ..." *Akzo NV v. U.S. International Trade Commission*, 1 U.S.P.Q. 2d 1241, 1245 (Fed. Cir. 1986). The Court of Appeals for the Federal Circuit has held that "a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a *single* prior art reference." *Verdegaal Bros. v. Union Oil of California,* 814 F.2d 628, 631 (Fed. Cir. 1988) (*emphasis added*).

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q.2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 U.S.P.Q.2d 1955, 1956 (Fed. Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992).

Further, the Federal Circuit has held that it is "impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir. 1992). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention" *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988).

Moreover, the Federal Circuit has held that "obvious to try" is not the proper standard under 35 U.S.C. §103. *Ex parte Goldgaber*, 41 U.S.P.Q.2d 1172, 1177 (Fed. Cir. 1996). "An-obvious-to-try situation exists when a general disclosure may pique the scientist curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claim result would be obtained if certain directions were pursued." *In re Eli Lilly and Co.*, 14 U.S.P.Q.2d 1741, 1743 (Fed. Cir. 1990).

2. The Rejections under 35 U.S.C. §102 and §103 Should Be Reversed Because the Patent Office Has Failed to Establish a *Prima Facie* Case of Anticipation and Obviousness

Appellants respectfully submit that the Patent Office has failed to overcome its *prima facie* burden with respect to the rejections of the claimed invention under 35 U.S.C. §102 or alternatively under §103. At the outset, the Patent Office has merely relied on a single reference in support of the rejections. Contrary to the Patent office's position, the anticipation rejection is improper. Further, Appellants do not believe that one skilled in the art would be inclined to modify same to arrive at the claimed invention.

a. The Claimed Lactic Bacterium Strain

Of the pending claims at issue, claims 1, 7, 9, 11, 12, 20 and 21 are the sole independent claims. Independent claim 1 recites a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus that has the ability to prevent colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses wherein the lactic acid bacterium strain is capable of growing in presence of up to 0.4% bile salts. Claim 7 recites a method for preparing an ingestable support that uses the biologically pure culture of lactic acid bacterium strain. Claim 9 recites a method for preparing an ingestable support material that uses a supernatant of a biologically pure culture of the lactic

acid bacterium strain. Claim 11 recites a method for treatment of a disorder associated with diarrhoea that includes administering to a patient having a disorder the biologically pure culture of lactic acid bacterium strain. Claim 12 recites a pharmaceutical composition that contains a biologically pure culture of lactic acid bacterium strain. Claim 20 recites a method for preventing a disorder associated with diarrhoea that includes administering the biologically pure culture of lactic acid bacterium strain. Claim 21 recites a food that includes a biologically pure culture of lactic acid bacterium strain.

The present invention relates to Lactobacilli strains that have the ability to prevent colonization of the intestine with both pathogenic bacteria and also against infection of intestinal epithelial cells by rotaviruses. The microorganisms of the present invention have been shown to exhibit a number of desirable properties. They are gram positive, catalase negative, NH_3 form arginine negative and carbon dioxide production negative. The microorganisms can produce L(+) lactic acid and are capable of growth in the presence of bile salts in a concentration of up to about 0.4%. See, Specification, p. 3, lines 21-25. Further, the lactic acid bacterium strains as claimed can effectively survive the passage through the gut, thus arriving in the intestine in an effectively live form so that the strains are readily capable of successfully colonizing the mucosa. Moreover, the claimed strains interact directly with the rotavirus receptors and synthesize metabolites and active compounds that are disadvantageous for the rotaviruses and pathogenic bacteria. See, Specification, p. 15, Example 5; and p. 16, Example 6. Therefore, the claimed strains provide inhibitory activity against colonization of the intestine with pathogenic bacteria casing diarrhea and infestation of intestinal cells with rotaviruses.

b. EP0861905 Fails to Disclose or Suggest the Claimed Invention

In contrast, Appellants believe that the cited art is distinguishable from the claimed invention. The primary focus of EP0861905 relates to a process which allows selection of Lactobacilli strains that are allegedly viable and resistant to technological treatments, such as freeze-drying or mixing with excipients. The strains are purportedly useful for the therapeutic and prophylactic treatment of disorders of the gastrointestinal system in humans. See, EP0861905, p. 2, line 38 and lines 46-48. For example, the Lactobacilli strains in EP0861905 are purportedly useful in the treatment of various disorders of the gastrointestinal tract, such as peristaltic disorders, gastroenteritis, heartburn, flatulence and diarrhea, particularly diarrhea

following the use of antibiotics (See, EP0861905, p.6, lines 7-8) or after an anti-tumor radiotherapy (See, EP0861905, p. 5, line 10).

Indeed, EP0861905 provides that Lactobacilli merely assist in reconstituting the micro-flora of the intestine or whether they are also providing an activity against some particular agents. In this regard, the clear focus of EP0861905 relates to strains as disclosed therein that oppose "pathogens", presumably through a lowering of the pH of the intestinal environment. See, EP0861905, p. 4, lines 33-35. As the term "pathogen" is not specifically defined therein, this term can therefore relate to parasites (like worms), fungi, bacteria, viruses, and the like, even to particles like prions or of inorganic nature. Thus, the cited art fails to recognize any specific significance to viruses, particularly rotaviruses, let alone the protection property against infection of intestinal epithelial cells by rotaviruses and/or preventing colonization of an intestine with pathogenic bacteria causing diarreha as claimed.

As previously discussed, the cited art provides that Lactobacilli oppose against pathogens through a lowering of the pH of the intestinal environment. See, EP0861905, p. 4, lines 31-35. Yet, it is not clear how the lowering of the pH effects any of the "pathogens" that may be contemplated as embraced by this term as discussed above. Indeed, rotaviruses exhibit a relatively high stability and will presumably not be effected by a relatively small lowering of the pH of the extracellular and intestinal environment caused by organic acids. Thus, a person skilled in the art would reasonably conclude that the strains as provided therein (which act by lowering the pH) are not capable of a prevention or treatment of an infection of the human intestine derived from rotaviruses in contrast to the claimed invention.

Further, nowhere does the sole cited reference mention pathogenic bacteria, let alone pathogenic bacteria causing diarrhea and prevention of colonizing thereof in the intestine. Clearly, the cited art further fails to disclose or suggest that micro-organisms can actively participate in the prevention or treatment of diarrhea derived from rotaviruses and simultaneously can protect against pathogenic bacteria causing diarrhea, are present in nature at all, and can be isolated in contrast to the claimed invention. Indeed, the claimed invention includes a biologically pure culture of a lactic acid bacterium strain, such as a supernatant thereof as further defined in claims 9, 12 and 21. Claim 1 further recites that the lactic acid bacterium strain is capable of growing in presence of up to 0.4% bile salts. Contrary to the Patent Office's

position, the single cited reference fails to recognize such a lactic acid bacterium strain as claimed that has the above-mentioned protective properties, that can arrive in the intestine in an essentially live form, that can adhere to the mucosa of the intestine and colonize it, and, once implanted in the mucosa (or even before), exert its beneficial effects, such as by interacting with cellular rotavirus receptors and secreting active metabolites into the environment.

Moreover, the emphasis of the cited art relates to a mixture of strains as disclosed therein (See, EP0861905, p. 5, lines 36-43) and thus effectively teaches against one strain that is effective for treatment purposes as claimed. In this regard, the EP0861905 reference provides that lactobacilli are typically administered orally, preferably as Paracasei and Salivarius mixtures. See, EP0861905, p. 5, lines 42-43. Indeed, the EXAMPLE in this reference merely relates to compositions that include both Lactobacillus Paracasei and Lactobacillus Salivarius. See, EP0861905, p. 6, lines 16-56. As previously discussed, Applicants have demonstrated that, one strain, namely Lactobacillus casei ST11 and deposited in accordance with the Budapest Treaty with the deposit number NCC 2461 (I-2116), was effective in preventing infection of human cells by rotaviruses. Moreover, this particular strain displayed desirable growing properties as may be shown by acidification in different media. The strain also displayed desirable performance with respect to the survival rate during storage at low temperatures of about 10°C. This makes it a suitable candidate for food stuff or pharmaceutical compositions, such as those stored at refrigerator conditions. (Specification, p. 7, lines 6-16).

Based on at least these differences, Appellants believe one skilled in the art would conclude that the claimed invention is distinguishable from the cited art. Again, the Patent Office has merely relied on a single reference in support of the anticipation or alternative obviousness rejections. Therefore, Appellants respectfully submit that the cited art fails to anticipate or render obvious the claimed invention. Accordingly, Appellants respectfully request that the rejections under 35 U.S.C. § 102 or alternatively § 103 be reversed.

IX. CONCLUSION

Appellants' claimed invention set forth in claims 1, 2 and 4-22 is neither taught nor suggested by the sole cited reference. The Patent Office has failed to establish a *prima facie* case of anticipation and alternative obviousness with respect to the rejection of the claimed invention.

Accordingly, Appellants respectfully submit that the anticipation and obviousness rejections are erroneous in law and in fact and should therefore be reversed by this Board.

Respectfully submitted,

BELL, BOYD & LLOYD LLC

BY _____

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Date: October 4, 2004

APPENDIX

Claim 1: A biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses wherein the lactic acid bacterium strain is capable of growing in presence of up to 0.4% bile salts.

Claim 2: The lactic acid bacterium strain according to claim 1, which is capable of adhering to an intestinal mucosa of a host organism and colonizing the intestinal mucosa.

Claim 4: The lactic acid bacterium strain according to claim 1 which is selected from the group consisting of Lactobacillus rhamnosus and Lactobacillus paracasei.

Claim 5: The lactic acid bacterium strain according to claim 4, which is Lactobacillus paracasei.

Claim 6: The Lactobacillus paracasei according to claim 5, which is Lactobacillus paracasei CNCM I-2116 (NCC 2461).

Claim 7: A method for preparing an ingestable support material comprising using a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 8: The method according to claim 7, wherein the lactic acid bacterium strain is contained in an ingestable support material in an amount from about 10^5 cfu / g to about 10^{12} cfu / g support material.

Claim 9: A method for preparing an ingestable support material comprising using a supernatant of a biologically pure culture of a lactic acid bacterium strain belonging to a genus

Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 10: The method according to claim 9, wherein the ingestable support material is a food composition selected from milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, and infant formulae.

Claim 11: A method for treatment of a disorder associated with diarrhoea comprising administering to a patient having the disorder associated with diarrhoea a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 12: A pharmaceutical composition containing a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of an intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses or a supernatant of a culture thereof.

Claim 13: The method according to claim 11, wherein the lactic acid bacterium strain is part of a composition which is selected from the group consisting of milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae, tablets, liquid bacterial suspensions, dried oral supplement, liquid oral supplement, dry tube feeding, and liquid tube feeding.

Claim 14: The pharmaceutical composition according to claim 12 wherein the lactic acid bacterium strain is capable of adhering to the intestinal mucosa of a host organism and essentially colonize it.

Claim 15: The pharmaceutical composition according to claim 12 wherein the lactic acid bacterium strain grows in the presence of up to 0.4 % bile salts.

Claim 16: The pharmaceutical composition according to claim 12 wherein the lactic acid bacterium strain is selected from the group consisting of Lactobacillus rhamnosus and Lactobacillus paracasei.

Claim 17: The pharmaceutical composition according to claim 16 wherein the lactic acid bacterium strain is Lactobacillus paracasei.

Claim 18: The pharmaceutical composition according to claim 17 wherein the lactic acid bacterium strain is Lactobacillus paracasei CNCM I-2116 (NCC 2461).

Claim 19: The method according to claim 7 wherein the ingestable support material is a food composition selected from the group consisting of milk, yogurt, curd, cheese, fermented milks, milk-based fermented products, ice-creams, fermented cereal based products, milk based product, and infant formulae.

Claim 20: A method for preventing a disorder associated with diarrhoea in a patient at risk of same comprising administering a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses.

Claim 21: A food comprising a biologically pure culture of lactic acid bacterium strain belonging to a genus Lactobacillus having the ability of preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and of preventing infection of intestinal epithelial cells by rotaviruses or a supernatant of a culture thereof.

Claim 22: The food according to claim 21 which is selected from the group consisting of milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae, tablets, liquid bacterial suspensions, dried oral supplement, wet oral supplement, and liquid tube feeding.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/936,542	01/18/2002	Roberto Reniero	112843-032	7122

24573 7590 04/06/2004

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EXAMINER	
WARE, DEBORAH K	
ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 04/06/2004

DUE: 7-6-04

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/936,542	RENIERO ET AL.	
	Examiner	Art Unit	
	Deborah K. Ware	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 January 2004* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-3,7-22 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-3,7-22 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-2 and 4-22 are presented for reconsideration on the merits.

The amendment filed January 2, 2004, has been received and entered.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-2 and 4-22 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicant's arguments filed January 2, 2004, have been fully considered but they are not persuasive. The deposit is noted and acknowledged, however, statement of availability as well as depository information in the specification has not been clearly set forth on the record. Thus, the rejection is reiterated below for Applicants' convenience.

Since the microorganism and strain is newly recited in the claims, it is essential to the invention recited in those claims. It must therefore be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. If the microorganism is not so obtainable or available, the requirements of 35 U.S.C. § 112 may be satisfied by a deposit of the microorganism. The specification does not disclose a repeatable process to obtain the microorganism and it is not apparent if the microorganism is readily available to the public. **It is noted that applicants have deposited the organism but there is no indication in the specification as to public availability**. If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by applicants, or a statement by an attorney of record over his or her signature and registration number, stating that the specific strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein.

If the deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 C.F.R. §§ 1.801-1.809, applicants may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that:

(a) during the pendency of this application, access to the invention will be afforded to the Commissioner upon request;

(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;

(c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer; and

(d) the deposit will be replaced if it should ever become inviable.

Applicant is directed to 37 CFR § 1.807(b) which states:

(b) A viability statement for each deposit of a biological material defined in paragraph (a) of this section not made under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure must be filed in the application and must contain:
(1) The name and address of the depository; (2) The name and address of the depositor; (3) The date of deposit;
(4) The identity of the deposit and the accession number given by the depository;
(5) The date of the viability test; (6) The procedures used to obtain a sample if the test is not done by the depository; and
(7) A statement that the deposit is capable of reproduction.

Applicant is also directed to 37 CFR § 1.809(d) which states:

(d) For each deposit made pursuant to these regulations, the specification shall contain:
(1) The accession number for the deposit;
(2) The date of the deposit; (3) A description of the deposited biological material sufficient to specifically identify it and to permit examination; and
(4) The name and address of the depository.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 2 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 2 is rendered grammatically unclear for the recitation of "colonize the intestinal mucosa", and it is suggested to change "colonize" to –colonizing--.

Double Patenting

Claims 1-2 and 4-22 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-22 of copending Application No. 09/936,489. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims encompass the identical Lactobacillus strain for which is used in various treatments and products for purposes of alleviating diarrhea, etc., albeit a different scope wherein the copending claims do not appear to read on treating and prevention pathogenic rotoviruses. However, such pathogenic organisms are well recognized in the prior art and Lactobacillus strains and varied species are well known to treat and prevent pathogenic microorganisms Therefore, one of ordinary skill in the art would have been motivated by the copending claims to provide for the claimed subject matter herein. The claims of the instant case are clearly obvious over the copending claims.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claims 1-2 and 4-22 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 & 3-23 of copending Application No. 09/936,543. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims

encompass the identical Lactobacillus strain for which is used in various treatments and products for purposes such as treating diarrhea, et c., albeit a different scope wherein the copending claims do not appear to read on preventing colonization of the intestine with pathogenic bacteria and rotoviruses; thus, the methods and products of the instant claims are very close and similar to the copending methods and products, however, they are not identical because of a difference in their scope. However, they are very similar and one of skill would have been motivated to provide for the instant methods and products from the copending methods and products. Therefore, the instant claims are prima facie obvious over the copending claims.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Applicant's arguments filed January 2, 2004, have been fully considered but they are not persuasive. A terminal disclaimer is necessary since no cases have been allowed and no terminal disclaimer has been approved.

Claim Rejections - 35 USC § 103

Claims 6 and 18 remain rejected under 35 U.S.C. 103(a) as being unpatentable over EP 0 861 905 A2, cited on the enclosed PTO-1449 Form for those reasons of record.

Claims are drawn to the Lactobacillus paracasei (L.p.) strain CNCM I-2116 and compositions thereof.

EP 0 861 905 A2 teaches L. p. strains and compositions thereof. Note the abstract and page 7, all lines.

The claims differ from the EP reference cited above in that the specific strain is not disclosed.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was filed to select for various strains having similar properties and expect successful results. Clearly these L. p. strains are well known in the art for similar uses, i.e. treating diarrhea and intestinal mucosa. Thus, one of skill in the art would have been motivated to select for other strains as well. A side by side comparison of the strains would show that they possess similar if not identical properties with respect to biochemical characteristics that they have in common. The claims are prima facie obvious over the cited art.

Applicant's arguments filed January 2, 2004, have been fully considered but they are not persuasive. The arguments that the cited references fails to provide any suggestion are noted, however, the reference teaches pathogens are treated with the microorganism strains and these are well known in the art to be bacteria and viruses and the like. Viruses are agents that cause diarrhea and the disclosed compositions and strains therefore, successfully treat diarrhea as disclosed by the cited reference. Therefore, viruses and pathogenic bacteria which also cause diarrhea which is disclosed to be treated by the disclosed composition are suggested by the reference. There would be an expected successful result with the claimed strains. Therefore, in the absence of persuasive evidence and a side by side comparison the claims are

rendered prima facie obvious. Lactobacillus strains are intrinsically capable of growth in bile salts (i.e. up to 0.4%).

Claim Rejections - 35 USC § 102

Claims 1-2 and 4-5, 7-17 and 19-22 are rejected under 35 U.S.C. 102(b) as being anticipated by EP 0 861 905 A2, discussed above, for reasons of record.

Claims are directed to varied uses of Lactic acid bacteria : treating intestinal muscosa, preparing products useful for the treatment, products containing the Lactobacillus strain.

The EP is discussed above.

The claims appear to be identical to the cited disclosure and are therefore considered to be anticipated by the teachings of the cited reference. Applicant's arguments filed January 2, 2004, have been fully considered but they are not persuasive for those reasons discussed above and those of record.

All claims fail to be patentably distinguishable over the state of the art discussed above. Therefore, the claims are properly rejected.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within

TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Deborah K. Ware whose telephone number is 308-4245. The examiner can normally be reached on 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on 308-4743. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 308-0196.

Deborah K. Ware
March 20, 2004

DAVID M. NAFF
PRIMARY EXAMINER
ART UNIT 1651

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 861 905 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
02.09.1998 Bulletin 1998/36

(21) Application number: **98103109.9**

(22) Date of filing: **23.02.1998**

(51) Int. Cl.[6]: **C12Q 1/10**, C12N 1/20, A61K 35/74, A23L 1/03 // A23C9/123 , (C12Q1/10, C12R1:225), (C12N1/20, C12R1:225)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **27.02.1997 IT MI970426**

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(54) Novel lactobacilli strains useful in the treatment of disorders of the gastrointestinal system

(57) Novel lactobacilli useful for the treatment of disorders of the gastrointestinal system, more particularly novel strains of Lactobacillus Paracasei and Lactobacillus Salivarius, the process for the preparation thereof, pharmaceutical compositions, dietary supplements and alimentary products containing them.

EP 0 861 905 A2

Printed by Xerox (UK) Business Services
2.16.3/3.4

Description

1. Field of the invention

The present invention relates to novel lactobacilli useful for the treatment of disorders of the gastrointestinal system, more particularly novel strains of Lactobacillus Paracasei and Lactobacillus Salivarius, the process for the preparation thereof, pharmaceutical compositions, dietary supplements and alimentary products (for example milk, yogurt or other dairy products) containing them.

Lactobacilli are widespread in nature. A number of species are present in fruits, vegetable and dairy products (fermented milk, cheese and yogurt), whereas only some strains can be found in the gastrointestinal and urogenital tracts of mammals and insects.

The beneficial activity of lactobacilli on gastrointestinal disorders has been known since ancient times and for a long time they were ingested with milk and dairy products.

2. Technological background

In most cases, known lactobacilli administered through diet or in other forms are not capable of crossing the gastric acid environment, and/or of resisting bile juices, thereby getting alive and viable into the intestinal action site and developing colonies numerous enough to exert significant therapeutical effects in short times.

The treatment of the gastrointestinal disorders thus performed usually requires prolonged administration times, and is often poorly effective in the most serious, persistent cases.

US 5,032,399 discloses a specific strain of Lactobacillus acidophilus recovered from the normal intestinal flora, which has been referred to as Lactobacillus GG and has subsequently been reclassified as Lactobacillus casei subs. rhamnosus, that is reported to have beneficial effects in the treatment of gastrointestinal disorders, to be stable to acids and bile juices and to be capable of adhering to the cells of human intestinal mucosa, of producing lactic acid and of growing effectively in vitro.

Recently, the Applicant has commercialized, under the name "Enterobacilli Proge Farm", a dietary supplement containing Lactobacilli recovered from intestinal flora, which is indicated for the treatment of the gastrointestinal system disorders. Said dietary supplement, likewise other commercial products containing Lactobacilli, has to be stored at low temperatures, specifically in refrigerator (at +4°C).

3. Problem to be solved

Various strains of Lactobacilli recovered from the intestinal environment, when used after selection on a laboratory scale, behave as good colonizers both in vitro and in vivo, exerting their activity in a short time and with satisfactory results.

However, for them to be suitable for a commercialisation on a large scale and for the use by consumers, said bacterial strains must undergo technological treatments (such as freeze-drying, mixing with excipients, subdivision in different dose units and formulation in pharmaceutical preparations or dietary supplements) which often adversely affect said strains, easily causing them to lose their peculiar probiotic properties and their original colonization capability.

A further technological problem is the poor stability to storage of the commercial Lactobacilli strains, which usually require a fridge storage, and which anyway easily lose their activity to a great extent.

4. Summary

The Applicant has now unexpectedly found a process which allows to select on an industrial scale Lactobacilli strains exceedingly viable and resistant to technological treatments, which strains are useful for the therapeutical, prophylactic and anyway probiotic treatments of disorders of the gastrointestinal system in humans, said process comprising incubating in a nutritive medium a previously freeze-dried, then rehydrated mixture containing a multiplicity of Lactobacilli strains, and selecting the first proliferating bacterial colonies, which develop not later than the first 12 hours, preferably within about 10 hours of incubation, in said nutritive medium at a temperature from about 30°C to about 40°C, preferably at 37°C.

The incubation step of the mixtures of freeze-dried strains is typically carried out on a plate, in a nutritive medium added with agar, preferably on an agarized MRS medium (de Man Rogosa Sharpe broth).

The formation of the colonies is suitably evidenced under an optical microscope, in particular by observing the reverse of the plate.

The mixture to be freeze-dried comes from the normal intestinal bacterial flora from an healthy human subject, and is typically obtained from biological samples containing a great number of bacterial species (for example intestinal

mucosa, faeces), recovered from human intestinal environment or from its contents by conventional techniques.

Before undergoing the process of the present invention, these samples are usually subjected to suitable pre-treatments according to conventional methods (for example physical pre-treatments, such as homogenization, and/or dilution in a suitable diluent, for example water).

The present process further comprises one or more pre-selection steps by incubation in a known nutritive medium selective for Lactobacilli, in the incubation conditions known for the development of Lactobacilli, from which bacterial mixtures highly enriched in Lactobacilli are obtained.

Said pre-selection step is preferably carried out before freeze-drying, on the biological sample preferably pre-treated as described above, to obtain bacterial mixtures which are subsequently subjected to freeze-drying and to the other selection steps according to the present invention, this step being typically effected in a liquid medium, for example a liquid MRS medium optionally added with other nutrients or useful substances, as specified hereinafter.

Further objects of the present invention are Lactobacilli strains and the bacterial cultures selected through the present process, the preparations or formulations containing the present Lactobacilli in combination with a pharmaceutically or anyway physiologically acceptable carrier, such as therapeutical and/or prophylactic compositions, dietary supplements, medical devices, alimentary products.

5. Detailed disclosure

The present process allows to select highly viable Lactobacilli, suitable for formulation on an industrial scale as pharmaceutical preparations or dietary supplements, which proved to be highly effective and advantageous in the treatment of various disorders of the gastrointestinal system.

According to an embodiment of the present invention, the mixtures of bacterial strains to be subjected to the present process come from samples of human faeces. According to another embodiment of the present invention, mixtures containing a great number of Lactobacilli belonging to human microintestinal flora are freeze-dried, rehydrated, incubated in a nutritive medium, the selected strains being incubated as described above, then further subjected to one or more of the following selection steps, preferably to all of them, in any order:

a) incubation in a nutritive medium containing bile, in a concentration of at least 0.1% weight/volume, and selection of the strains proliferating in said medium;

b) incubation in a nutritive acid medium, having pH lower than or equal to 5.5 (more preferably lower than 3.5), and selection of the strains proliferating in said medium;

c) incubation in a nutritive medium containing cells of the intestinal mucosa of the subject to treat, in particular humans, and selection of the bacteria capable of adhering to the cells at an average rate corresponding to the adhesion of at least 50 bacteria per each intestinal cell in an incubation time up to 5 minutes;

d) incubation of the strains in a nutritive medium and selection of the bacteria which produce at least 3.5 mEq of lactic acid per 10^{10} CFU (Colony Forming Units) at 37°C in a time of 24 hours;

e) incubation of the strains in a nutritive medium, and selection of the bacterial strains which proliferate at 37°C with a generation time of less than one hour, more preferably of about 45 minutes.

The selection steps indicated above as a) to e) are typically carried out in the operative conditions described in US 5,032,399 and in the corresponding EP 199,535, whose contents is herein incorporated by reference, with the difference that mixtures of microorganisms from a previous selection, deriving from the collection of microorganisms capable of differentiating in a nutritive medium incubated with previously freeze-dried and rehydrated strains, are subjected to steps a) to d).

More specifically, the present process comprises the following steps:

1) incubation of a mixture of microorganisms containing a multiplicity of Lactobacilli in a medium selective for Lactobacilli, and collection of the thus developed bacterial cells;

2) freeze-drying of the bacterial cells obtained from the previous step, preferably in the presence of one or more additives for freeze-drying;

3) rehydration of the freeze-dried mixture from the previous step;

4) incubation of the resulting freeze-dried and rehydrated mixture in a nutritive medium, preferably in a medium selective for Lactobacilli, and collection of the cells developed not later than the first 12 hours, preferably within about 10 hours of incubation at 37°C in said nutritive medium.

The cultures of Lactobacilli recovered according to the present process can be sometimes contaminated by traces of bacterial strains belonging to other species from microintestinal flora, without departing from the scope of the present invention.

The medium selective for Lactobacilli is, for example, an MRS culture broth (de Man Rogosa Sharpe broth, Oxoid), commercialized by DIFCO and described in J. Applied Bacteriol. (1960), 23, 130, having the composition reported in the above cited US 5,032,399 and EP-B199,535, or other selective medium known for this purpose, optionally added with other nutrients or other substances. For example an MRS medium added with bile salts (for example in a 0.5% weight/volume concentration on the total of the broth) can be used.

The incubation in a medium selective for Lactobacilli according to step 1) is typically carried out in a liquid medium.

The incubation of the freeze-dried, rehydrated bacterial strains, as in step 4) is typically effected on agarized plates, preferably in a selective medium for Lactobacilli, although, alternatively, it can be effected in a different nutritive medium conventionally known for their development.

The above cited incubation steps of lactobacilli are typically carried out at a temperature from 30°C to 40°C, preferably at 37°C, preferably under mildly anaerobic atmosphere.

The freeze-drying is preferably carried out in the presence of additives known as freeze-drying protectives, for example powder skimmed milk (in amounts for example of about 10-12% by weight on the basis of the Lactobacilli to be freeze-dried), saccharose, glycerol, or anyway operating according to alternative, conventional freeze-drying techniques.

The present process allows to select Lactobacilli, such as the strains deposited at the Institut Pasteur, as reported in the following, having high resistance to the technological treatments, particularly high viability and high stability to storage at temperatures of about +15°C/+20°C, and moreover, of one or more of the following advantageous and surprising characteristics: ability to produce lactic acid in a substantially pure stereomeric L-(+) form, or anyway in an amount by far higher than the isomer D(-), resistance to various antibiotics, in particular insensitiveness to tetracyclins but sensitivity to other antibiotics, in particular rifampicin and erythromycin.

According to a further advantageous characteristic, the lactobacilli obtained according to the present process are not capable of transferring their resistance to tetracyclins to other microorganisms, including the pathogenic ones.

In particular, the lactobacilli according to the present invention have the further features:

- stability to acids;
- stability to bile juices;
- capability of adhering to the cells of the intestinal mucosa;
- capability of growing effectively in vitro.

The capability of producing only, or anyway in a high prevalence, L-(+)-Lactic acid, i.e. the L(+) isomer, which is not metabolized by the body, and can remain for a long time in the intestinal lumen, where it exerts its effects, is extremely advantageous, in that the lactic acid produced by lactobacilli is at least partly responsible for the beneficial effects of the colonization of the gastrointestinal tract by lactobacilli, thus opposing pathogens presumably through a lowering of the pH of the intestinal environment.

The sensitivity to some types of antibiotics evidenced for the lactobacilli according to the present invention is an important, advantageous feature. It is in fact known that "cross-resistance" phenomena to antibiotics can occur through migration of plasmidic material from one microorganism to another. Therefore, the administration of Lactobacilli resistant to all of the antibiotics is potentially dangerous for the body, in that pathogens strains resistant to all of the antibiotics could be selected.

It is therefore important that the Lactic bacterium administered for the treatment of disorders of the gastrointestinal system be sensitive to at least some antibiotics, so as to have suitable therapeutical weapons available, should the above mentioned cross-resistance occur.

Contrary to the Lactobacilli recovered by the present process, various known Lactobacilli strains, among which the Lactic bacterium GG disclosed in US 5,032,399, turned out to be insensitive to all of the antibiotics and thus disadvantageous from this point of view.

The selection according to the process of the invention can be operated periodically so as to isolate and use strains with different lysotypia, keeping however probiotic characteristics , such as:

- ability to reach the action site remaining integer;
- ability to settle at the gastrointestinal level;
- production of L-(+)-Lactic acid.

The possibility of selecting strains with different lysotypia allows to assure the productivity and effectiveness of the fermentation process and/or a safe colonization in the intestine even in the presence of phagi, which are capable of phagocytizing and destroying lactobacilli both in the fermentation environment and in the intestinal lumen, thus jeopardizing the production of lactobacilli or the therapeutical or dietetic treatments making use of them.

Moreover, the Lactobacilli strains of the invention are unexpectedly endowed with a higher stability compared with

those obtained by the known processes, as they can be stored for long times at temperatures above 4°C, in particular at about +15°C/+20°C. For example, after about 18-24 months at about 22°C, the decrease in viable cells observed is only of 1 logarithmic unit.

Thanks to this property, the preparations of the invention show amounts of lactobacilli alive of only about 1 logarithm less of cells per unitary dose, compared with the number of cells alive at time zero, even after months from the commercialisation, contrary to what observed for various commercial compositions of the prior art, which show a high decrease in the alive cells compared with those specified at the time of the packaging, and which therefore are often poorly effective from the therapeutical point of view.

In fact, the treatment with Lactobacilli according to the present invention yields favourable results, such as the nearly complete remission of diarrhoea, even when severe and obstinate, due for example to antitumour radiotherapy, in short times i.e. about 1-2 days, which is markedly less than the time required with the lactobacilli of the prior art. The rapidity of action can be at least partly ascribed to the high storage stability shown by the Lactobacilli of the invention.

More precisely, the process of the invention allowed to select novel Lactobacilli strains belonging to the Paracasei and Salivarius species, in particular 3 strains deposited under the Budapest Treaty at the "Collection Nationale de Cultures de Microorganismes" (CNCM) of the Institut Pasteur (25, Rue du Docteur Roux, 75724 PARIS CEDEX 15), under the following accession numbers:

- Lactic bacterium PF1S, deposited on April 3, 1996, under the accession number CNCM I-1687, with the taxonomic designation Lactobacillus Paracasei;
- Lactic bacterium PF2P, deposited on April 3, 1996, under the accession number CNCM I-1688, with the taxonomic designation Lactobacillus Paracasei;
- Lactic bacterium PF1S1, deposited on December 6, 1996, under the accession number CNCM I-1794, with the taxonomic designation Lactobacillus Salivarius.

The three Lactobacilli strains Paracasei and Salivarius above identified by their accession numbers produce exclusively L(+) Lactic acid, are sensitive to erythromycin and rifampicin and insensitive to tetracyclines, are stable to acids, resisting at pH 3.5 for 30 minutes at 40°C.

In particular, the L. Paracasei strains, particularly the Lactic bacterium PF2P CNCM I-1688, are insensitive to aztreonam, cefotixina, vancomycin and tetracycline, and sensitive to erythromycin, rifampicin and cefotaxime, and they ferment ribose; and Salivarius strains, such as the PF1S1 CNCM I-1794 strain, are insensitive to cefotaxime and tetracycline, and sensitive to erythromycin and rifampicin.

The lactobacilli according to the present invention can be administered as such or they can be added to a pharmaceutically or anyway physiologically acceptable carrier, to obtain various types of preparations or formulations, such as pharmaceutical compositions, dietary supplements or alimentary products, in particular dairy products (such as milk, yogurt), which are a further object of the present invention.

The present preparations or formulations contain at least one Lactic bacterium according to the present invention, preferably mixtures of at least one Lactic bacterium of the species Paracasei or Salivarius, in particular in effective amounts for the therapeutical, prophylactic or probiotic treatments desired and can be prepared, for example, according to conventional techniques.

The carrier can contain excipients, additives, diluents or other useful substances, provided they are pharmacologically or physiologically acceptable.

For the treatment of the disorders of the gastrointestinal system according to the invention, lactobacilli are typically administered orally, preferably as Paracasei and Salivarius mixtures.

The daily effective amount for humans is generally comprised from 10^7 to about 2×10^9 Lactobacilli daily, for an adult (weighing on the average 50-70 Kg), or from 10^6 to 10^8 Lactobacilli for infants.

Typical preparations for the treatment of disorders of the gastrointestinal system according to the present invention contain for example 10^6 to 3×10^9 UFC of Lactobacilli for unitary dose.

Preferably, the preparations according to the invention contain lactobacilli in the freeze-dried form.

For this purpose, aqueous mixtures containing lactobacilli, and preferably at least one freeze-drying additive, are typically freeze-dried in the conditions reported above for the process of selection of lactobacilli according to the invention, or according to known procedures.

Preferably, for the purposes of the invention, lactobacilli, typically in freeze-dried form, are combined with excipients or additives selected from: vitamins (such as vitamins of the group B, for example vitamin B1 and B2, vitamin C and mixtures thereof), hydrocarbons (for example sweeteners, such as saccharose, and maltodextrins) and silicium dioxide, preferably in admixture, for example mixtures of vitamins B1, B2, C, saccharose, maltodextrins and silicium dioxide.

Vitamins are present in amounts depending on the dosage typical of the vitamin, for example about 0.1-5 mg of vitamin B1 or B2 for 10^9 Lactobacilli and about 5-20 mg of Vitamin C for 10^9 Lactobacilli; hydrocarbons are present for example in amounts of 500-1000 mg for 10^9 Lactobacilli (for example about 500-800 mg of saccharose for 10^9 Lacto-

bacilli, preferably combined with about 100-300 mg of maltodextrins for 10^9 Lactobacilli); silicium dioxide is present for example in amounts of about 0.5-5.0 mg for 10^9 Lactobacilli.

Preferably, in the final product, the freeze-dried product containing the Lactobacilli is packaged in sachets in paper/aluminium/polyethylene foil.

The lactobacilli of the invention are valuable for the treatment of various disorders of the gastrointestinal tract, for example those due to, but non limited to, alterations of the equilibrium of the intestinal flora, in particular diarrhoea and constipation, for example specific and aspecific diarrhoea (specially diarrhoea following the use of antibiotics), chronic intestinal inflammations, colitis, flatulence, heartburn, gastroenteritis, aphthous stomatitis, peristaltic disorders, other conditions of gastrointestinal impaired function and/or dysmicrobism consequent to surgery, kidney or liver disorders, radiotherapy, dietary unbalances, emotional stress, ageing or immune system disorders.

The Lactobacilli of the invention are moreover effective in the treatment of the irritable bowel. This is a particularly unexpected result in that, whereas lactobacilli are known to colonize mainly the distal intestine, they are not known to be capable of colonize colon in large amounts.

Some exemplary embodiments of the invention are reported in the following.

EXAMPLE

Composition for the oral use, in freeze-dried form, contained in sachets of paper/aluminium/polyethylene foil, each containing:

- Lactobacillus Paracasei PF2P, in amounts of at least 10^9 UFC;
- Lactobacillus Salivarius PF1S1, in amounts of at least 10^9 UFC;

Vitamin B1	mg 0.6;
Vitamin B2	mg 0.5;
Vitamin C	mg 20.0;
Saccharose	mg 1417.8;
Maltodextrins	mg 400.0;
Cream flavour	mg 50.0;
Silicium dioxide	mg 5.0;

or

- Lactobacillus Paracasei PF1S, in amounts of at least 10^9 UFC;
- Lactobacillus Salivarius PF1S1, in amounts of at least 10^9 UFC;

Vitamin B1	mg 0.6;
Vitamin B2	mg 0.5;
Vitamin C	mg 20.0;
Saccharose	mg 1417.8;
Maltodextrins	mg 400.0;
Cream flavour	mg 50.0;
Silicium dioxide	mg 5.0.

The following tables show the fermentation profiles of the three Lactobacilli strains deposited at the Institut Pasteur, obtained by means of the API 50CHL system.

TABLE 1

The strain of L. Paracasei PF1S-CNCM I-1687 ferments the following hydrocarbons:

L-Arabinose, Ribose, Galactose, D-Glucose, D-Fructose, D-Mannose, L-Sorbose, Rhamnose, Dulcitol, Inositol, Mannitol, Sorbitol, α-Methyl-D-Mannoside, α-Methyl-D-Glucoside, N-Acetylglucosamine, Amygdalin, Arbutin, Esculin, Salicin, Cellobiose, Maltose, Lactose, Melibiose, Saccharose, Threalose, Inulin, Melicitose, β-Gentiobiose, D-Turanose, D-Tagatose.

TABLE 2

L. Paracasei PF2P-CNCM I-1688 ferments the following hydrocarbons:

L-Arabinose, Ribose, Galactose, D-Glucose, D-Fructose, D-Mannose, L-Sorbose, Rhamnose, Dulcitol, Inositol, Mannitol, Sorbitol, α-Methyl-D-Mannoside, α-Methyl-D-Glucoside, N-Acetylglucosamine, Amygdalin, Arbutin, Esculin, Salicin, Cellobiose, Maltose, Lactose, Melibiose, Saccharose, Trealose, Inulin, Melicitose, a-Gentiobiose, D-Turanose, D-Tagatose.

TABLE 3

L. Salivarius strain PF1S1-CNCM I-1794 ferments the following hydrocarbons:

Galactose, D-Glucose, D-Fructose, D-Mannose, Mannitol, Sorbitol, N-Acetylglucosamine, Maltose, Lactose, Melibiose Saccharose, Trealose, D-Raffinose, Xylitol, D-Arabitol.

Claims

1. A process for the selection of Lactobacilli strains useful in the treatment of disorders of the gastrointestinal tract in humans, comprising the incubation in a nutritive medium of a mixture containing a multiplicity of Lactobacilli strains, coming from the normal human intestinal bacterial flora, previously freeze-dried and then rehydrated, followed by the selection of the colonies which develop not later than the first 12 hours of incubation in said nutritive medium at a temperature from 30°C to 40°C.

2. A process according to claim 1, comprising the following steps:

   1) incubation of a mixture of microorganisms containing a multiplicity of Lactobacilli in a selective medium for Lactobacilli, and collection of the bacterial cells thus developed;
   2) freeze-drying of the bacterial cells from the previous step, preferably in the presence of one or more freeze-drying additives;
   3) rehydration of the freeze-dried mixture from the previous step;
   4) incubation of the resulting freeze-dried and rehydrated mixture, in a medium for selective Lactobacilli or in other nutritive mediums, and collection of the cells developed not later than the first 12 hours of incubation at 37°C in said nutritive medium.

3. A process according to claim 1 or 2, in which the collection of the cells developed after incubation of the above freeze-dried and rehydrated mixture is carried out within 10 hours of incubation at 37°C.

4. A process according to claim 1 or 2, in which the incubation is carried out in an MRS culture broth.

5. A process according to claims 1 to 4, in which the incubation of the above freeze-dried and rehydrated mixture is carried out on plates, in a nutritive medium added with agar.

6. A process according to claim 1 or 2, in which the incubation steps are carried out under mildly anaerobic atmos-

phere.

7. A process according to claim 1 or 2, in which the freeze-drying is carried out in the presence of freeze-drying additives selected from powder skimmed milk, saccharose and glycerol.

8. A process according to claim 1 or 2, in which the incubation of the above freeze-dried and rehydrated strains is followed by one or more of the following selection steps, carried out in any order:

a) incubation in a nutritive medium containing bile, in concentrations of at least 0.1% weight/volume, and selection of the strains proliferating in said medium;
b) incubation in a nutritive acid medium, having pH lower than or equal to 5.5 (more preferably lower than 3.5), and selection of the strains proliferating in said medium;
c) incubation in a nutritive medium containing cells of the intestinal mucosa of the subject to treat, and selection of the bacteria capable of adhering to the cells at an average rate corresponding to the adhesion of at least 50 bacteria for each intestinal cell in an incubation time lower than or equal to 5 minutes;
d) incubation of the strains in a nutritive medium and selection of the bacteria which produce at least 3.5 mEq of lactic acid per 10^{10} UFC (Colony Forming Units) at 37°C in a time of 24 hours;
e) incubation of the strains in a nutritive medium, and selection of the bacterial strains which proliferate within a generation time at 37°C of less than one hour.

9. A culture of bacterial strains belonging to the Lactobacillus species, obtainable according to the process as defined in claims 1 to 8.

10. A culture of a bacterial strain belonging to the Lactobacillus species, characterized by: stability at storage at 22°C corresponding to a decrease in alive cells of 1 logarithm after 18-24 months; ability to produce lactic acid in a substantially pure L-(+) stereomeric form; resistance to tetracyclines, combined with sensitivity to rifampicin and erythromycin; no transmissibility of the resistance to tetracyclines.

11. A culture of Lactobacilli according to claim 10, further characterized by the following properties: ability to adhere to the cells of the human intestinal mucosa in an average amount of at least 50 bacteria for each intestinal cell in an incubation time up to 5 minutes; ability to produce at least 3.5 mEq of lactic acid per 101° UFC (Colony Forming Units) at 37°C within a time of 24 hours; ability to reproduce in a nutritive medium within a generation time at 37°C of less than one hour.

12. A culture according to claim 9 or 10, in which the Lactobacilli strains derive from the normal microintestinal flora.

13. A culture according to claim 9 or 10, in which the bacterial strains are the Lactobacilli strains Paracasei or Salivarius.

14. A culture according to claim 9 or 10, selected from the following cultures deposited at the CNCM collection of the Institut Pasteur:

- Lactobacillus Paracasei, CNCM I-1687;
- Lactobacillus Paracasei, CNCM I-1688;
- Lactobacillus Salivarius, CNCM I-1794.

15. A preparation for therapeutical, prophylactic or probiotic use in the treatment of disorders of the gastrointestinal system in humans, containing an effective amount of at least one Lactic bacterium strain as defined in each of claims 9 to 14, in combination with a pharmaceutically or physiologically acceptable carrier.

16. A preparation according to claim 15, selected from pharmaceutical compositions, dietary supplements, and alimentary products.

17. A preparation according to claim 15, for the oral use.

18. A preparation according to claim 15, containing at least one Lactic bacterium of the Paracasei, Salivarius species, or mixtures thereof.

19. A preparation according to claim 15, in which said Lactic bacterium strain is in the freeze-dried form.

20. A preparation according to claim 15, selected from pharmaceutical compositions and dietary supplements, in which lactobacilli are combined with excipients or additives selected from vitamins, hydrocarbons and silicium dioxide.

21. A preparation according to claim 15, containing vitamin B1 and B2, vitamin C and mixtures thereof, saccharose, maltodextrins and silicium dioxide.

22. The use of a culture containing a Lactic bacterium strain as defined in each of claims 9 to 14, in the treatment of disorders of the gastrointestinal system in humans.

23. The use according to claim 22, in which said disorders are selected from the group consisting of specific and aspecific diarrhoea, diarrhoea following the use of antibiotics, constipation, chronic intestinal inflammations, colitis, flatulence, heartburn, gastroenteritis, aphthous stomatitis, peristaltic disorders, dysmscrobism conditions, impaired gastrointestinal functionality consequent to conditions selected from surgery, kidney and liver disorders, radiotherapy, dietary unbalances, emotional stress, ageing and immune system disorders.

24. The use according to claim 22, in which the disorder is irritable bowel.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/936,542	01/18/2002	Roberto Reniero	112843-032	7122

29157 7590 07/26/2004

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EXAMINER	
WARE, DEBORAH K	
ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 07/26/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Advisory Action	Application No.	Applicant(s)	
	09/936,542	RENIERO ET AL.	
	Examiner	Art Unit	
	Deborah K. Ware	1651	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 07 June 2004 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

a) ☐ The period for reply expires _____months from the mailing date of the final rejection.

b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection. ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1.☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2.☐ The proposed amendment(s) will not be entered because:

(a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);

(b) ☐ they raise the issue of new matter (see Note below);

(c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

(d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: _____.

3.☒ Applicant's reply has overcome the following rejection(s): 35 USC 112, first and second paragraphs. See Attachment A..

4.☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5.☒ The a)☐ affidavit, b)☐ exhibit, or c)☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because: *see Attachment A*.

6.☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7.☒ For purposes of Appeal, the proposed amendment(s) a)☐ will not be entered or b)☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: *None*.

Claim(s) objected to: *None*.

Claim(s) rejected: *1-2 and 4-22*.

Claim(s) withdrawn from consideration: *N/A*.

8.☐ The drawing correction filed on _____ is a)☐ approved or b)☐ disapproved by the Examiner.

9.☐ Note the attached Information Disclosure Statement(s)(PTO-1449) Paper No(s). _____.

10.☐ Other: _____

Attachment A

Claims 1-2 and 4-22 are presented in the after final of June 7, 2004.

The rejections under 35 USC 112, first and second paragraphs are removed.

Further, it is acknowledged that Applicants will file terminal disclaimers to overcome the obvious double patenting rejections of record, however, the rejections are maintained until the terminal disclaimers have been received.

The response to the 35 USC 102/103 rejection is also noted. While Applicants do acknowledge the Lactobacilli strains of the prior art to be viable and resistant to freeze drying, they fail to note that strains may be selected for which are also resistant to phagi (i.e. bacteriophage or viruses), note page 4, lines 54-55. Thus, while the prior art strains are useful against pathogens including bacteria and viruses, they are useful for colonizing the gut or gastrointestinal tract, note pages 2, lines 39-40 and 6, lines 10-15. Furthermore, the cited prior art clearly recognizes a protection property against infection of intestinal track and hence cells thereof by viruses may include rotaviruses. This is an inherent property of the selected lactobacilli strains of the cited prior art.

Also the cited prior art teaches that the selected Lactobacilli strains have a higher stability compared with others, note bridging pages 4-5, lines 55-60 and lines 1-5, respectively. In addition, the cited prior art teaches that thanks to these properties, the strains of the selected Lactobacilli are alive upon administering to the gastrointestinal tract and remedy diarrhea, note page 5, lines 4-10. Once the Lactobacilli strains colonize as disclosed by the cited prior art they will inherently interact with cellular rotavirus receptors and secrete metabolites, such as lactic acid. Further, the argument

that the prior art teaches a mixture of strains is noted, however, the prior art merely establishes that more than one strain may be employed in the preparations as does Applicants claimed invention. Therefore, the differences for which Applicants have presented are not believed by the examiner to be pertinent since there appear to be no differences. Thus, the rejections are maintained for reasons of record with the exception of the ones noted above as being removed.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Deborah K. Ware whose telephone number is 571-272-0924. The examiner can normally be reached on 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Deborah K. Ware
July 10, 2004

...D M. ...
...MARY EXAMINE...
...RT UNIT 1651